Measurement of partition coefficients by various centrifugal partition chromatographic techniques

A comparative evaluation

NABIL EL TAYAR, RUEY-SHIUAN TSAI, PHILIPPE VALLAT, COSIMO ALTOMARE and
BERNARD TESTA*

*Institut de Chimie Thérapeutique, Ecole de Pharmacie, Université de Lausanne, BEP, CH-1015 Lausanne
(Switzerland)*

ABSTRACT

Using published and previously unpublished data, the present paper compares the value of four centrifugal partition chromatography systems for measuring partition coefficients. The best results (broad applicability, log P range -3 to $+3$, precision, effectiveness) were obtained with the Ito multilayer coil separator-extractor and the horizontal flow-through multilayer centrifugal partition chromatography model. Excellent correlations were found with published log P values obtained by the shake-flask method.

INTRODUCTION

Lipophilicity, as expressed by the partition coefficient P, has since the pioneering work of Meyer [1] and Overton [2] become a major physicochemical parameter in medicinal chemistry. This property is often a determinant of the pharmacokinetic and pharmacodynamic behaviour of xenobiotics [3–6]. Thermodynamically, partition coefficient is defined as a constant relating the concentration of a solute in two immiscible phases at equilibrium [7,8]. A number of experimental models are currently used to simulate partition processes in biological systems and to determine lipophilicity. The "shake-flask" (SF) method, using water and a poorly misible organic solvent, is the technique most widely used for measuring partition coefficients [9,10]. 1-Octanol–water is universally accepted as the standard biphasic solvent system [9], but other solvents are of value, for example in understanding the relative contribution of hydrogen-bonding capacity to lipophilicity [11 14]. However, despite its value, the SF method suffers from a number of practical limitations, such as lack of precision, solute stability or volatility, solute impurities, formation of microemulsions, time consumption, etc., as previously discussed by Dearden and Bresnen [15].

Partition chromatography has been explored as an alternative means for measuring lipophilicity. In particular, chromatographic retention parameters obtained by

reversed-phase high-performance liquid chromatography (RP-HPLC) have become increasingly popular in replacing the 1-octanol–water partition coefficients measured by the SF method [16–19]. However, the assumption that the mechanism of retention in RP-HPLC should be similar to the mechanism of partitioning in a 1-octanol–water system is an oversimplification. Indeed, the chemically bonded solid phases are expected, owing to the restricted mobility of the bonded alkyl chains and the presence of a solid support with a non-negligible proportion of residual silanol groups, to display a partitioning behaviour different from that of a true liquid such as 1-octanol [20–22].

Recently, centrifugal counter-current chromatography, also known as centrifugal partition chromatography (CPC), has been explored as a novel technique for measuring liquid–liquid partition coefficients [23–30]. This is a unique form of liquid–liquid partition chromatography that eliminates the need for a solid support; in other words, adsorption is precluded by the absence of a solid support, and solute retention depends only on its partition coefficient. Two poorly miscible liquids are used as the stationary and mobile phase. A centrifugal force maintains the stationary phase, while the mobile phase is pumped through the system. During the last decade, various CPC systems have been developed, such as the flow-through multilayer coil planet centrifuge [31,32], the horizontal flow-through multilayer coil planet centrifuge [33,34], the toroidal coil planet centrifuge [35,36], and the multichannel cartridges CPC [37,38].

In this paper we attempt to asses the potential application of four different centrifugal counter-current chromatographic techniques, namely multichannel cartridges CPC, toroidal coil planet centrifuge, flow-through multilayer coil planet centrifuge and horizontal flow-through multilayer coil planet centrifuge, in measuring partition coefficients.

THEORY AND GENERAL CONSIDERATIONS

Centrifugal counter-current chromatography is a liquid–liquid chromatographic technique resembling to some extent droplet counter-current chromatography (DCCC). In DCCC, the stationary phase is retained in a series of vertical narrow-bore tubes, while the mobile phase, depending upon its density, is pumped through the system in the ascending or descending mode in the form of small droplets [39–41]. CPC differs from DCCC in that centrifugal and/or Archimedian screw forces maintain the stationary phase, while the mobile phase is pumped through. These features allow high partition efficiency and large retention capability of the stationary phase under a high flow-rate of the mobile phase.

In a recent review [42], Ito presented the historical background, development and mechanisms of distribution of stationary and mobile phases in the CPC coil, classifying CPC systems into two types, namely hydrostatic equilibrium systems and hydrodynamic equilibrium systems. Briefly, the hydrostatic systems use a stationary coil, for example PTFE tubing such as those used in the toroidal coil planet centrifuge chromatograph [35,36] or multichannel cartridges such as those used in the Sanki CPC chromatograph [37,38]. Measurements begin with filling the coil with the stationary phase; and then the mobile phase, depending on its density, in introduced at the head or the tail of the coil, displacing nearly half the volume of the stationary phase in the coil. Hence, solutes introduced at the inlet of the coil are subjected to a

continuous partitioning process between the two phases. In this system, the retention of the stationary phase and the distribution of stationary and mobile phases in the coil are governed mainly by the centrifugal force.

In hydrodynamic systems such as the flow-through multilayer coil planet centrifuge [31,32] or the horizontal flow-through multilayer coil planet centrifuge [33,34], the rotation of the coiled column around its own axis creates an Archimedean screw force which, in combination with a revolutionary centrifugal force towards the centre of the centrifuge, allows a continuous mixing of the two phases while retaining a high proportion of the stationary phase. Using a stroboscope, Ito [42] observed that under high centrifugal forces and flow-rates the mixing zone, located near the centre of the centrifuge where the centrifugal force is weakest, travels towards one end of the coil. This indicates that the two phases are subjected to a typical partitioning process of repetitive mixing and settling at a high rate of over 13 times per second while the mobile phase is steadily passing through the stationary phase. These features provide a high partition efficiency and a high retention capability of the stationary phase under a high flow-rate of the mobile phase in comparison with the hydrostatic systems. For more detail, the reader is referred to the excellent review of Ito [42].

In these systems, the partition coefficient (log P^{CPC}) is defined as the ratio of solute concentration in the stationary phase and in the mobile phase. In solvent systems using water as mobile phase, the partition coefficient is calculated as:

$$\log P^{CPC} = \log [(V_R - V_M)/V_S] \tag{1}$$

where V_R is the retention volume of the solute, V_M is the dead volume (mobile phase volume) and V_S is the stationary phase volume. In our laboratory, the dead volume (V_M) was determined using potassium dichromate as the non-retained compound. Using the organic solvent as mobile phase, the partition coefficient is readily calculated as:

$$\log P^{PCP} = \log [(V_S)/V_R - V_M] \tag{2}$$

In this case, the dead volume (V_M) was determined using anthracene as the non-retained compound.

What distinguishes the toroidal coil centrifuge from other CPC apparatus is that movement of coloured solutes can be observed continuously through the transparent coil using a stroboscopic light source [35]. This feature allows the measurement of highly lipophilic or highly hydrophilic solutes which take a very long time to elute. In such cases, the retention time (t_R) can be calculated when the solute is still far from the column outlet by measuring the distance of the position of the centre of the solute band (X_t) at time t as:

$$t_R = t(X_R/X_t) \tag{3}$$

where X_R is the distance of the circumference of the support around which the coil is wound. Hence, V_R can be calculated from the predetermined flow-rate of the mobile phase [31].

EXPERIMENTAL

Multichannel cartridges CPC apparatus

A Model CPC-LLN chromatograph (Sanki Engineering, Kyoto, Japan) connected to a 2238 Uvicord II detector operating at 254 nm (LKB, Bromma, Sweden) and a 600 chart recorder (W + W Scientific, Basle, Switzerland) was used. The chromatograph was fitted with twelve Type 250W cartridges (total volume 250 ml) placed in the rotor of a centrifuge [38]. Each cartridge is composed of four poly(chlorotrifluoroethylene) plates and five PTFE sheets. The rotor is thermostated in a constant-temperature box, and all experiments were performed at 30°C. Solvent was delivered by a Sanki constant-flow pump (Model LBP-V, Sanki Engineering). The apparatus was first packed with the stationary phase and then the mobile phase was pumped through. Depending upon the density of the two phases, the eluent was pumped in a head-to-tail or tail-to-head mode. A systematic determination of the dead volume is very important in the Sanki CPC-LLN model because of continuous "bleeding" of the stationary phase [25].

Toroidal coil planet centrifuge

The original design of the toroidal coil centrifuge has already been described [30]. A helical column is mounted in the periphery of the column container located on the top of the rotor. The helical column was prepared by winding PTFE tubing (0.55 mm I.D.) (Zeus Industrial Products, Raritan, NJ, USA) around a nylon tube (110 cm × 4 mm O.D.) to make *ca.* 830 turns with a total capacity of 4.0 ml. The movement of coloured solutes was observed by stroboscopic illumination with a visible light source.

In a previous study [30], 1-octanol and 0.1 M phosphate aqueous buffer were used as stationary and mobile phases, respectively. The rotational speed was adjusted to 1000 rpm and the flow-rate was 0.4 ml/min.

Flow-through multilayer coil planet centrifuge

A preparative coil (2.6 mm I.D., 370 ml volume capacity) was fitted in an Ito multilayer coil separator-extractor (P.C. Inc., Kim Place, Potomac, MD, USA). For commuting between "head" and "tail" ends of the coil, an SRV-4 four-way valve (Pharmacia, Uppsala, Sweden) was installed. The solutes were injected through a Lobar six-port valve injector (Merck, Darmstadt, Germany) with a 2.5-ml loop mounted. A Uvikon 725 UV detector (Kontron, Zurich, Switzerland) equipped with a QS 1.000 80-μl UV cell was used. The chromatograms were recorded with a Model 3392A integrator (Hewlett-Packard, Meyrin, Switzerland). A more detailed description of the apparatus was reported by Slacanin *et al.* [43].

1-Octanol–aqueous buffer and *n*-heptane aqueous buffer solvent systems were used. The rotation speed of the rotor was about 1000 rpm. Depending upon the estimated distribution coefficient values, the flow-rate and the volume ratio of stationary and mobile phases were selected so that reasonable and precise retention time of solutes could be obtained. Thus, a flow-rate of 0.5 ml/min and a 36:1 volume ratio of stationary and mobile phases were employed for hydrophilic compounds with log P values smaller than -2.3. The other experimental details were as previously reported [29].

Horizontal flow-through multilayer CPC

The horizontal flow-through multilayer CPC model, CCC-1000 (Pharma-Tech Research, Baltimore, MD, USA) consists of three columns, each being helically wound with five layers of PTFE tubing (3.00 mm I.D., 3.94 mm O.D., volume capacity 115 ml). A Kontron Model 432 UV detector and a Model 420 HPLC pump (Kontron) were used. The chromatograms were recorded with a 3392A integrator (Hewlett-Packard). The experimental procedure is the same as for the Ito multilayer coil separator extractor.

Chemicals

All compounds were of highest available purity and were obtained from different pharmaceutical and chemical companies. Analytical grade 1-octanol, cyclohexane, n-heptane and 3-morpholinopropane sulfonic acid (MPS) were purchased from Merck.

RESULTS AND DISCUSSION

Comparison of partition coefficients measured by CPC instruments with literature values measured by the shake-flak method

Multichannel cartridge CPC. Terada *et al.* [23,24] have measured the partition coefficients of various organic compounds using the Sanki CPC model and a solvent system consisting of 1-octanol- n-hexane (20:80)–water solvent system. A good linear relationship between (1-octanol–n-hexane (20:80)-water partition coefficients (log P^{CPC}) and 1-octanol–water partition coefficients measured by the SF method (log P^{SF}) was found. The reason for using octanol hexane mixtures as the organic solvent was to decrease the high viscosity of 1-octanol, which caused pressure problems. However, the decrease in polarity of the octanol resulting from the addition of n-hexane is known to influence dramatically the mechanism of partitioning compared with the 1-octanol water solvent systems [44]. Berthod and Armstrong [25,26] showed that the Sanki CPC model can be used directly to determine 1-octanol water partition coefficients over a log P range of -2 to 2 by changing the number of cartridges in the rotor.

A recent study [28] has also compared the partition coefficients obtained by the Sanki CPC model and the SF method. Two solvent systems were employed, namely 1-hexanol–aqueous buffer (0.02 M MPS, pH 7.4) and cyclohexane–aqueous buffer (0.02 M MPS, pH 7.4). The aqueous and organic phases were mutually saturated. Preliminary studies using a 1-octanol–aqueous buffer and n-hexane aqueous buffer systems were not successful, probably because of the high viscosity of 1-octanol and the low density and/or viscosity of n-hexane, respectively. In the 1-hexanol aqueous buffer system, 1-hexanol was used as the mobile phase and aqueous buffer as the stationary phase. The flow-rate was 1.8 ml/min and the pump pressure was 55 kg/cm^2 at a rotation rate of 500 rpm. In the cyclohexane aqueous buffer system, water was used as the mobile phase and cyclohexane as the stationary phase; the flow-rate was 2.4 ml/min and the pump pressure was 50 kg/cm^2 at a rotation of 700 rpm. In 1-hexanol water and cyclohexane–water systems, a good reproducibility of partition coefficient measurements was obtained (S.D. $<$ 4%) by the Sanki CPC model. 1-Hexanol–water and cyclohexane water partition coeffcients expressed as log $P^{CPC}_{hexanol}$ and log $P^{CPC}_{cyclohex}$, respectively, are reported in Table I. Table I also reports literature

partition coefficents obtained by the SF method in the 1-octanol water and hexane water solvent systems and expressed as log P_{oct}^{SF} and log P_{hex}^{SF} [45], respectively. Good linear relationships between partition coefficients obtained by the Sanki CPC model (log P^{CPC}) and partition coefficients measured by the SF method (log P^{SF}) were obtained as follows:

$$\log P_{oct}^{SF} = 1.25(\pm 0.16) \log P_{hexanol}^{CPC} - 0.25(\pm 0.16)\ (n = 20;\ r = 0.968;\ s = 0.15)\ (4)$$

$$\log P_{hex}^{SF} = 1.05(\pm 0.31) \log P_{cyclohex}^{CPC} - 0.21(\pm 0.29)\ (n = 8;\ r = 0.959;\ s = 0.26)\ (5)$$

where n is the number of compounds, r is the correlation coefficient, and s is the standard deviation of regression. The values in parentheses are the 95% confidence limits of the regression coefficients. Eqns. 4 and 5 indicate that multichannel cartridges CPC is a useful method for measuring partition coefficients. However, the narrow range of measurable lipophilicites, the continuous "bleeding" of the stationary phase, the high pressure and the resulting breakage of tubing limit the usefulness of this technique.

Toroidal coil CPC. Toroidal coil CPC has been used by some to measure partition coefficients of a few coloured compounds [30]. The log P^{CPC} values measured by this technique are reported at the end of Table II. This technique proved its potential

TABLE I

PARTITION COEFFICIENTS OF VARIOUS ORGANIC COMPOUNDS MEASURED BY MULTICHANNEL CARTRIDGES CPC USING 1-HEXANOL WATER AND CYCLOHEXANE WATER SOLVENT SYSTEMS

Solute	Log $P_{hexanol}^{CPC}$	Log $P_{octanol}^{SF\,a}$	Log $P_{cyclohexane}^{CPC}$	Log $P_{hexane}^{SF\,a}$
Benzenesulphonamide	0.44	0.31	−2.28	−[b]
Phenylmethylsulphoxide	0.60	0.55	−1.29	−
Phenylmethylsulphone	0.69	0.49	−0.59	−
Benzamide	0.74	0.64	−1.92	−2.35
Acetanilide	0.99	1.16	−1.31	−1.80
Aniline	1.03	0.90	0.12	−0.05
Benzyl alcohol	1.08	1.10	−0.46	−0.76
Phenylethanol	−[b]	−	0.01	−0.39
Phenol	1.34	1.47	−0.69	−0.89
Phenyl acetate	1.32	1.49	−	−
Nitrobenzene	1.45	1.86	−	−
4-Aminophenol	0.11	0.04	−1.62	−
2-Aminophenol	0.70	0.52	−1.02	−
4-Nitroaniline	1.49	1.39	−0.92	−0.62
3-Nitroaniline	1.35	1.37	−	−
2-Nitroaniline	1.47	1.83	0.42	0.21
4-Pyridylmethanol	0.22	0.06	−	−
4-Pyridylethanol	0.30	0.10	−	−
4-Pyridylpropanol	0.80	0.58	−	−
4-Pyridylbutanol	1.01	0.90	−	−
4-Pyridylpentanol	1.47	1.39	−	−

[a] Taken from ref. 45.

[b] Not determined.

TABLE II

PARTITION COEFFICIENTS OF VARIOUS ORGANIC COMPOUNDS MEASURED BY THE ITO MULTILAYER COIL SEPARATOR EXTRACTOR (UNLESS OTHERWISE INDICATED) USING 1-OCTANOL–WATER AND *n*-HEPTANE–WATER SOLVENT SYSTEMS

Solute	Log $P^{CPC}_{octanol}$	Log $P^{SFa}_{octanol}$	Log $P^{CPC}_{heptane}$	Log $P^{SFa}_{heptane}$
Phenol	$-$[b]	1.46	-0.82	-0.70
2-Chlorophenol	2.05	2.14	$-$	$-$
4-Chlorophenol	$-$	2.39	-0.12	-0.11
2-Nitrophenol	1.68[c]	1.72[c]	$-$	$-$
3-Nitrophenol	1.74[c]	1.52[c]	-1.23	-1.40
4-Nitrophenol	1.77[c]	1.38[c]	-2.11	-2.00
2-Aminophenol	$-$	0.62	-2.46	-2.51
3-Aminophenol	0.15	0.17	$-$	$-$
3-Methoxyphenol	$-$	1.58	-0.88	-0.72
4-Methoxyphenol	$-$	1.34	-1.03	-1.16
4-Methylphenol	$-$	1.94	-0.19	-0.35
2,6-Difluorophenol	1.46[c]	$-$	$-$	$-$
Aniline	$-$	0.90	0.03	0.04
2-Nitroaniline	$-$	1.85	0.31	0.25
3-Nitroaniline	$-$	1.37	-0.46	-0.56
4-Nitroaniline	1.30[d]	1.39	-1.09	-1.13
4-Chloroaniline	2.01	1.88	$-$	$-$
N-Methylaniline	1.69	1.66	$-$	$-$
N,N-Dimethylaniline	2.32	2.31	2.40	2.23
m-Phenylenediamine	$-$	$-$	-2.51	-2.60
p-Phenylenediamine	$-$	-0.30	-3.01	-3.00
Benzene	2.05	2.13	2.37	2.29
Fluorobenzene	2.26	2.27	2.46	2.45
Chlorobenzene	$-$	2.81	2.99	2.95
Nitrobenzene	$-$	1.85	1.53	1.43
Toluene	2.54	2.73	2.85	2.85
Benzaldehyde	$-$	1.48	1.12	1.05
Benzamide	0.65	0.64	$-$	$-$
4-Fluorobenzamide	0.96	0.91	$-$	$-$
2-Chlorobenzamide	1.35	$-$	$-$	$-$
2-Bromobenzamide	0.71	0.73	$-$	$-$
Benzyl alcohol	1.22	1.10	-0.62	-0.55
2-Fluorobenzyl alcohol	1.31	$-$	$-$	$-$
4-Fluorobenzyl alcohol	1.36	$-$	$-$	$-$
2,6-Difluorobenzyl alcohol	1.12	$-$	$-$	$-$
Benzylamine	1.15	1.09	$-$	$-$
4-Chlorobenzoic acid	2.66	2.65	$-$	$-$
4-Bromobenzoic acid	2.74	2.86	$-$	$-$
4-Iodobenzoic acid	3.00	3.02	$-$	$-$
4-Hydroxybenzoic acid	1.56	1.58	$-$	$-$
Anisole	$-$	2.11	2.15	2.10
Acetophenone	$-$	1.58	1.20	1.14
2-Chloroacetanilide	1.35	1.28	$-$	$-$
2-Naphthol	2.85	2.84	$-$	$-$
Pyridine	$-$	0.65	-0.31	-0.30
2-Aminopyridine	0.51	0.49	$-$	$-$
4-Pyridylmethanol	-0.04	-0.02	$-$	$-$
4-Pyridylpropanol	0.59	0.60	$-$	$-$

TABLE II (*continued*)

Solute	Log $P_{\text{octanol}}^{\text{CPC}}$	Log $P_{\text{octanol}}^{\text{SF}a}$	Log $P_{\text{heptane}}^{\text{CPC}}$	Log $P_{\text{heptane}}^{\text{SF}a}$
Catechol	−	−	− 2.72	2.85
Caffeine	−	− 0.07	− 2.21	− 2.18
Pentobarbital	−	2.07	− 1.22	− 1.30
Secobarbital	−	1.97	− 0.99	− 1.00
Hexobarbital	−	1.49	− 0.57	− 0.70
Morphine	0.84	0.76	−	−
Sulpiride	0.52	0.58	−	−
Sulphamerazine	0.07	0.14	−	−
Sulphathiazole	0.04	0.05	−	−
Sulphanilamide	− 0.85	− 0.72	−	−
BzSA[e]	0.33	0.35	− 2.54	−
4-Methyl-BzSA	0.83	0.80	− 2.42	−
3-Methyl-BzSA	0.85	0.90	− 2.35	−
4-Chloro-BzSA	−	1.10	− 2.27	−
3-Chloro-BzSA	−	1.20	− 2.21	−
4-Bromo-BzSA	−	1.38	− 2.17	−
3-Bromo-BzSA	−	1.39	− 2.04	−
4-Iodo-BzSA	1.77	1.59	− 1.91	−
3-Iodo-BzSA	1.58	1.62	− 1.91	−
4-Isopropyl-BzSA	1.96	1.75	− 1.37	−
3-Isopropyl-BzSA	1.96	1.70	− 1.41	−
4-Phenyl-BzSA	2.60	2.28	− 1.24	−
4-Cyano-BzSA	0.40	0.22	− 2.54	−
3-Cyano-BzSA	0.27	0.26	− 2.33	−
4-Acetyl-BzSA	0.31	0.24	− 2.74	−
3-Acetyl-BzSA	0.23	0.25	− 2.74	−
4-Methoxy-BzSA	0.48	0.45	− 2.71	−
3-Methoxy-BzSA	0.65	0.57	− 2.33	−
4-Nitro-BzSA	0.72	0.75	− 2.96	−
3-Nitro-BzSA	0.60	0.56	− 2.98	−
4-Butyloxy-BzSA	2.19	2.09	− 1.12	−
3-Butyloxy-BzSA	2.09	2.10	− 0.94	−
4-Hexyloxy-BzSA	2.83	2.93	− 0.41	−
4-Sulphonamido-BzSA	− 0.70	− 0.96	−	−
3-Sulphonamido-BzSA	− 0.56	− 0.46	−	−
4-Carboxyamido-BzSA	− 0.66	− 0.79	−	−
3-Carboxyamido-BzSA	− 0.55	− 0.80	−	−
4-Amino-BzSA	0.64	− 0.62	−	−
3-Amino.BzSA	− 0.38	− 0.28	−	−
4-Methylsulphonamido-BzSA	− 0.73	− 0.36	−	−
4-Acetanilido-BzSA	− 0.10	0.00	−	−
4-Hydroxy-BzSA	− 0.06	− 0.06	−	−
4-ADS[f]	1.84	1.76	− 0.97	−
4'-Methyl-4-ADS	−	2.40	− 0.49	−
2',4'-Dimethyl-4-ADS	2.69	2.73	− 0.63	−
4'-Fluoro-4-ADS	2.17	2.01	0.76	−
4'-Chloro-4-ADS	−	2.57	− 0.91	−
4'-Bromo-4-ADS	3.15	2.85	− 0.44	−
4'-Cyano-4-ADS	−	1.63	− 1.43	−
4'-Acetyl-4-ADS	−	1.67	− 1.34	−
4'-Methoxycarbonyl-4-ADS	2.13	2.25	− 1.18	−
4'-Methoxy-4-ADS	1.99	1.96	− 0.98	−

TABLE II (*continued*)

Solute	Log $P^{CPC}_{octanol}$	Log $P^{SFa}_{octanol}$	Log $P^{CPC}_{heptane}$	Log $P^{SFa}_{heptane}$
2',4'-Dimethoxy-4-ADS	1.65	1.63	− 1.50	−
2',4',6'-Trimethoxy-4-ADS	−	1.03	− 2.27	−
4'-Nitro-4-ADS	2.03	2.13	− 1.00	−
2',4'-Dinitro-4-ADS	1.84	2.04	− 1.22	−
4'-N,N-Diethylamino-4-ADS	−	1.44	− 2.03	−
2'-Amino-4-ADS	1.75	1.56	− 1.70	−
2',4'-Diamino-4-ADS	0.38	0.38	−	−
4'-N,N-Dimethylamino-4-ADS	1.93	2.04	− 1.19	−
4'-N-Ethylamino-4-ADS	−	1.98	− 1.38	−
4'-N-Hydroxylamino-4-ADS	−	0.88	− 2.30	−
2',4',6'-Trihydroxy-4-ADS	1.75	1.53	−	−
Cytidine[g]	− 2.32	− 2.10	−	−
Adenosine[g]	− 1.03	− 0.98	−	−
Inosine[g]	− 2.11	− 2.00	−	−
Uridine[g]	− 1.98	− 1.89	−	−
Cytosine[g]	− 1.51	− 1.73	−	−
Guanosine[g]	− 1.94	− 0.92	−	−
Thymidine[g]	− 1.19	− 1.10	−	−
Uracil[g]	− 1.14	− 1.07	−	−
Thymine[g]	− 0.61	− 0.62	−	−
5-Fluorouracil[g]	− 1.01	− 0.95	−	−
Adenine[g]	− 0.16	− 0.09	−	−
Glycine[g]	− 3.00[h]	− 3.00[i]	−	−
Alanine[g]	− 2.77[h]	− 2.74[i]	−	−
Proline[g]	− 2.62[h]	− 2.54[i]	−	−
Phenylalanine[g]	− 1.44[h]	− 1.52[i]	−	−
Tryptophane[g]	− 1.15[h]	− 1.11[i]	−	−
Tyrosine[g]	− 2.11[h]	− 2.42[i]	−	−
Pararosaniline	− 0.21[d]	−	−	−
Crystal violet	0.51[d]	−	−	−
Sudan III	2.11[d]	−	−	−
Phenol red	3.02[d]	−	−	−
MPTP[j]	2.71	2.61	−	−
4'-amino-MPTP	1.48	−	−	−
MPP[+k]	− 2.28	− 1.04	−	−
4'-Methyl-MPP[+]	− 1.90	−	−	−

[a] Taken from ref. 45.

[b] Not determined.

[c] Log distribution coefficient (log D) at pH 7.4.

[d] Measured by toroidal coil CPC 31.

[e] BzSA = benzenesulphonamide.

[f] 4-ADS = 4-aminodiphenylsulphone.

[g] Measured by horizontal flow-through CPC.

[h] Log distribution coefficient (log D) at isoelectric point.

[i] Log distribution coefficient (log D) at pH 7.0.

[j] MPTP = 1-phenyl-4-phenyl-1,2,3,6-tetrahydropyridine.

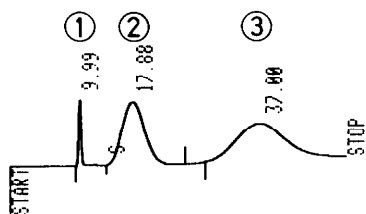[k] MPP[+] = 1-methyl-4-phenyl-pyridinium iodide.

Fig. 1. A chromatogram of a mixture of 4-amino-BzSA and 4-acetanilido-BzSA using the flow-through multilayer CPC apparatus. The abscissa is the retention time in minutes and the ordinate is the UV spectral absorbance. Phosphate buffer (pH 7.4) and 1-octanol were used as the mobile and stationary phases, respectively. Peaks: 1 = potassium dichromate; 2 = 4-amino-BzSA; 3 = 4-acetanilido-BzSA.

to calculate the partition coefficient of highly lipophilic or hydrophilic coloured compounds from their movement observed in the transparent coil under stroboscopic light. Thus, the retention time of highly lipophilic or hydrophilic solutes, which take a very long time to elute, can be calculated using eqn. 3 when the solute is still far from the column outlet. Unfortunately, no literature log P^{SF} values are available for comparison except p-nitroaniline (log P^{SF} = 1.39; log P^{CPC} = 1.30). The most obvious limitation of the method is solute detection, which in the present state of development
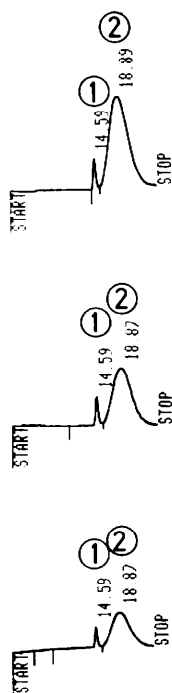
Fig. 2. A triplicate measurement of 5-fluorouracil at three different concentrations using the horizontal flow-through multilayer CPC. The abscissa is the retention time in minutes and the ordinate is the UV spectral absorbance. The volume of stationary and mobile phases is 262.5 ml and 87.5 ml, respectively. Aqueous MPS buffer (pH 7.4) was used as the mobile phase and 1-octanol as the stationary phase; the flow-rate was adjusted to 6 ml min. Peaks: 1 = potassium dichromate; 2 = 5-fluorouracil.
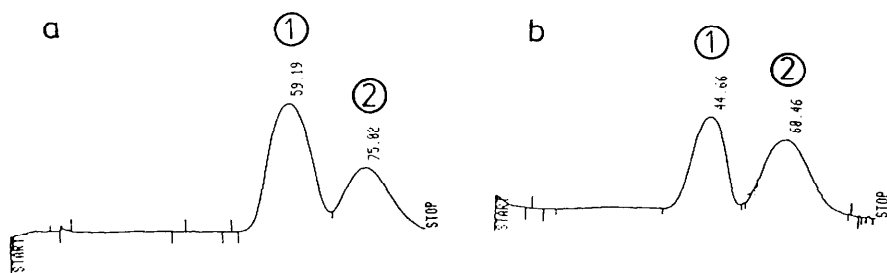
Fig. 3. Chromatograms of MPTP at different stationary and mobile phase volume ratios: (a) 5:1 and (b) 7:1. The abscissa is the retention time in minutes and the ordinate is the UV spectral absorbance. The distribution coefficient of MPTP was exactly the same (log D^{CPC} = 1.29). The flow-through multilayer CPC apparatus was used, 1-octanol being the mobile phase and phosphate buffer (pH 7.4) the stationary phase; the flow-rate was 1.00 ml/min. Peaks: 1 = anthracene; 2 = MPTP.

is restricted to coloured compounds. However, we believe that the method could be extended to all UV-active solutes by using a stroboscopic UV light source.

   Flow-through CPC and horizontal flow-through CPC. 1-Octanol–aqueous buffer and *n*-heptane–aqueous buffer partition coefficients (log P_{oct}^{CPC} and log P_{hep}^{CPC}) measured by the Ito multilayer coil separator-extractor and the horizontal flow-through CPC (CCC-1000 model), are reported in Table II. The two techniques gave very satisfactory results. However, the Ito multilayer coil separator-extractore instrument is limited to a rotational speed of 800 rpm and the coil should be accurately balanced. The range of measurable log *P* values is − 3.0 to + 3.0 in the two solvent systems, and the average time of a triplicate measurement is about 2 h. The average time can be reduced by measuring a mixture of compounds, provided that their peaks are fully separated as illustrated in Fig. 1. An excellent reproducibility of partition coefficients was obtained (S.D. < 1%) by both the Ito multilayer coil separator-extractor and the horizontal flow-through CPC, as illustrated in Fig. 2. Fig. 2 also reveals that the retention time of solutes was constant in the usual concentration range. Furthermore, the precision of measurements by these techniques is not affected by changing the
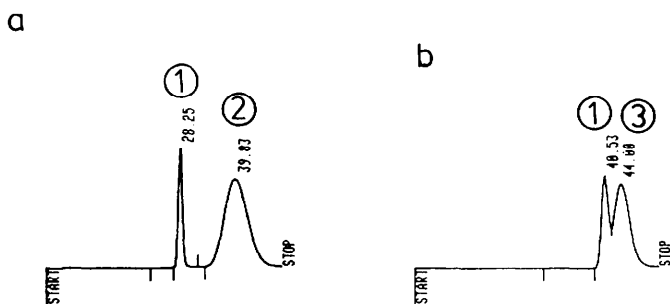

Fig. 4. Chromatograms of (a) 4′-amino-MPTP (log D^{CPC} = − 0.19) and (b) 4′-methyl-MPP$^+$ (log D^{CPC} = − 1.90) using the flow-through multilayer CPC apparatus. The abscissa is the retention time in minutes and the ordinate is the UV spectral absorbance. Phosphate buffer (pH 7.4) and 1-octanol were used as the mobile and stationary phase, respectively. The flow-rate was (a) 8.00 ml/min, and (b) 1.10 ml/min. Peaks: 1 = potassium dichromate; 2 = 4′-amino-MPTP; 3 = 4′-methyl-MPP$^+$.
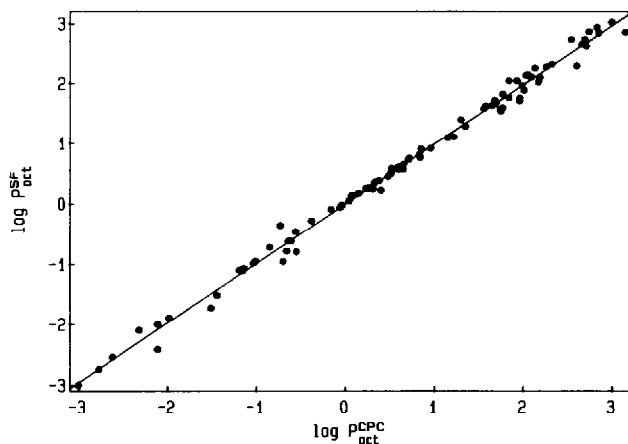
Fig. 5. Linear relationship between 1-octanol-water partition coefficients measured by the CPC and SF methods (eqn. 6).

flow-rate of the mobile phase and/or the volume ratio of stationary and mobile phases, as shown in Fig. 3. Both the aqueous and organic solvent can be employed as mobile phases depending on the lipophilicity of solutes. In both cases, very stable baselines, as shown in Fig. 3 and 4, were observed.

Excellent correlations were found with literature partition coefficients measured by the shake-flask method (log P^{SF}), as demonstrated in Figs. 5 and 6 and eqns. 6 and 7:

$$\log P^{SF}_{oct} = 0.99(\pm 0.01) \log P^{CPC}_{oct} - 0.01(\pm 0.02) \quad (n=89; \ r=0.997; \ s=0.12) \quad (6)$$

$$\log P^{SF}_{hep} = 0.99(\pm 0.01) \log P^{CPC}_{hep} - 0.04(\pm 0.02) \quad (n=30; \ r=0.999; \ s=0.08) \quad (7)$$
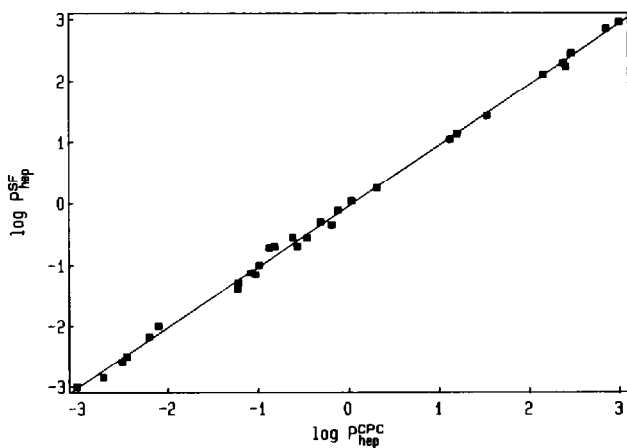

Fig. 6. Linear relationship between n-heptane-water partition coefficients measured by the CPC and SF methods (eqn. 7).

Guanosine and MPP$^+$ were excluded from eqn. 6, the deviations being most probably the result of some inherent limitations of the shake-flask method. These equations (6 and 7) clearly indicate that the same partitioning mechanism is operative in both CPC and SF methods, the slopes being equal to one and the intercepts to zero. In addition, these equations prove the potential applicability of CPC for measuring the partition coefficient of various drugs and many chemical compounds of chemical or biological interest (Table II).

CONCLUSIONS

CPC is demonstrated in this study to be a valuable alternative to the SF and RP-HPLC methods for measuring lipophilicity. Indeed, CPC combines the advantages of the SF method (ability to obtain genuine partition coefficients, possibility of using a variety of solvent systems) with those of RP-HPLC (rapidity, reproducibility, decreased interference by impurities). In our experience, solutes of log P values ranging from -3 to $+3$ can be measured in triplicate in about 2 h, and the results for a large number of compounds correlated very well with published log P values obtained by the SF method. The experimental conditions reported here result from a two-year effort in optimization, but more progress is to expected as CPC systems become dedicated to the measurement of partition coefficients.

ACKNOWLEDGEMENTS

REFERENCES

1   H. Meyer, *Arch. Exp. Pathol. Pharmakol.*, 42 (1899) 109.
2   E. Z. Overton, *Phys. Chem.*, 22 (1897) 189.
3   H. Kubinyi, in E. Jucker (Editor), *Progress in Drug Research*, Vol. 23, Birkhäuser, Basle, 1979, p. 97.
4   J. C. Dearden and P. K. Mays, *J. Pharm. Pharmacol.*, 37 (1985) 70P.
5   R. F. Rekker, *Farmaco Ed. Sci.*, 34 (1979) 346.
6   J. M. Mayer and H. van de Waterbeemd, *Environ. Health Perspec.*, 61 (1985) 295.
7   M. Berthelot and E. Jungfleisch, *Ann. Chim. Phys.*, 26 (1872) 396.
8   S. C. Valvani, S. H. Yalkowsky and T. J. Roseman, *J. Pharm. Sci.*, 70 (1981) 502.
9   A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
10 A. Hersey, A. P. Hill, R. M. Hyde and D. J. Livingstone, *Quant. Struct.-Act. Relat.*, 8 (1989) 288.
11 P. Seiler, *Eur. J. Med. Chem.*, 9 (1974) 473.
12 T. Fujita, T. Nishioka and M. Nakajima, *J. Med. Chem.*, 20 (1977) 1071.
13 M. Gryllaki, H. van de Waterbeemd, B. Testa, N. El Tayar, J. M. Mayer and P.-A. Carrupt, *Int. J. Pharm.*, 51 (1989) 95.
14 N. El Tayar, R.-S. Tsai, P.-A. Carrupt, B. Testa and A. Leo, *J. Pharm. Sci.*, June 1991.
15 J. C. Dearden and G. M. Bresnen, *Quant. Struc.-Act. Relat.*, 7 (1988) 133.
16 N. El Tayar, H. van de Waterbeemd and B. Testa, *J. Chromatogr.*, 320 (1985) 305.
17 T. Braumann, *J. Chromatogr.*, 373 (1986) 191.
18 R. Kaliszan, in J. D. Winefordner (Editor), *Qantitative Structure–Chromatographic Retention Relationships*, Wiley-Interscience, New York, 1987.
19 D. J. Minik, J. J. Sabatka and D. A. Brent, *J. Liq. Chromatogr.*, 10 (1987) 2565.
20 A. Nahum and Cs. Horvath, *J. Chromatogr.*, 203 (1981) 53.

21 E. Bayer and A. Paulus, J. Chromatogr., 400 (1987) 1.
22 N. El Tayar, A. Tsantili-Kakoulidou, T. Röthlisberger, B. Testa and J. Gal., J. Chromatogr., 439 (1988) 237.
23 H. Terada, Y. Kosuge, N. Nakaya, W. Murayama, Y. Nunogaki and K.-I. Nunogaki, Chem. Pharm. Bull., 35 (1987) 5010.
24 H. Terada, Y. Kosuge, W. Murayama, N. Nakaya, Y. Nunogaki and K.-I. Nunogaki, J. Chromatogr., 400 (1987) 343.
25 A. Berthod and D. W. Armstrong, J. Liq. Chromatogr., 11 (1988) 547.
26 A. Berthod and D. W. Armstrong, J. Liq. Chromatogr., 11 (1988) 567.
27 C. Altomare, R.-S. Tsai, N. El Tayar, B. Testa, A. Carotti, S. Cellamare and P. G. De Benedetti, J. Pharm. Pharmacol., 43 (1991) 191.
28 N. El Tayar, A. Marston, A. Bechalany, K. Hostettmann and B. Testa, J. Chromatogr., 469 (1989) 91.
29 P. Vallat, N. El Tayar, B. Testa, J. Slacanin, A. Marston and K. Hostettmann, J. Chromatogr., 504 (1990) 411.
30 R.-S. Tsai, N. El Tayar, B. Testa and Y. Ito, J. Chromatogr., 538 (1991) 119.
31 Y. Ito and R. L. Bowman, J. Chromatogr., 147 (1978) 221.
32 Y. Ito, J. Chromatogr., 188 (1980) 33.
33 Y. Ito, J. Chromatogr., 207 (1981) 161.
34 Y. Ito and H. Oka, J. Chromatogr., 457 (1988) 393.
35 Y. Ito and R. L. Bowman, Anal. Biochem., 85 (1978) 614.
36 Y. Ito, J. Chromatogr., 192 (1980) 75.
37 W. Murayama, T. Kobayashi, Y. Kosuge, H. Yano, Y. Nunogaki and K. Nunogaki, J. Chromatogr., 239 (1982) 649.
38 D. Armstrong, G. L. Bertrand and A. Berthod, Anal. Chem., 60 (1988) 2513.
39 T. Tanimura, J. J. Pisano, Y. Ito and R. L. Bowman, Science (Washington, D.C.), 169 (1970) 54.
40 Y. Ito, R. L. Bowman, J. Chromatogr. Sci., 8 (1970) 315.
41 W. D. Conway and Y. Ito, LC Mag., 2 (1984) 368.
42 Y. Ito, CRC Crit. Rev. Anal. Chem., 17 (1986) 65.
43 I. Slacanin, A. Marston and K. Hostettmann, J. Chromatogr., 482 (1989) 234.
44 S. Okada, H. Nakahara, C. Yomota and K. Mochida, Chem. Pharm. Bull., 33 (1985) 4.
45 C. Hansch and A. Leo, The Pomona Medicinal Chemistry Project, Pomona College, Claremont, CA, 1983.